Simultaneous Physiologically Based Pharmacokinetic (PBPK) Modeling of Parent and Active Metabolites to Investigate Complex CYP3A4 Drug-Drug Interaction Potential: A Case Example of Midostaurin[§]

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ABSTRACT

Midostaurin (PKC412) is being investigated for the treatment of acute myeloid leukemia (AML) and advanced systemic mastocytosis (advSM). It is extensively metabolized by CYP3A4 to form two major active metabolites, CGP52421 and CGP62221. In vitro and clinical drug-drug interaction (DDI) studies indicated that midostaurin and its metabolites are substrates, reversible and time-dependent inhibitors, and inducers of CYP3A4. A simultaneous pharmacokinetic model of parent and active metabolites was initially developed by incorporating data from in vitro, preclinical, and clinical pharmacokinetic studies in healthy volunteers and in patients with AML or advSM. The model reasonably predicted changes in midostaurin exposure after single-dose administration with ketoconazole (a 5.8-fold predicted versus 6.1-fold observed increase) and rifampicin (90% predicted versus 94% observed reduction) as well as changes in midazolam exposure (1.0

predicted versus 1.2 observed ratio) after daily dosing of midostaurin for 4 days. The qualified model was then applied to predict the DDI effect with other CYP3A4 inhibitors or inducers and the DDI potential with midazolam under steady-state conditions. The simulated midazolam area under the curve ratio of 0.54 and an accompanying observed 1.9-fold increase in the CYP3A4 activity of biomarker 4 β -hydroxycholesterol indicated a weak-to-moderate CYP3A4 induction by midostaurin and its metabolites at steady state in patients with advSM. In conclusion, a simultaneous parent-and-active-metabolite modeling approach allowed predictions under steady-state conditions that were not possible to achieve in healthy subjects. Furthermore, endogenous biomarker data enabled evaluation of the net effect of midostaurin and its metabolites on CYP3A4 activity at steady state and increased confidence in DDI predictions.

Introduction

Midostaurin is a potent kinase inhibitor of FMS- like tyrosine kinase 3 (FLT3), KIT (including D816V and Y mutants), platelet-derived growth factor receptor β , vascular endothelial growth factor receptor 2, fibroblast growth factor receptor, and protein kinase C. FLT3 mutations occur in approximately 30% of patients with acute myeloid leukemia (AML). Midostaurin is equally active against FLT3 with internal tandem duplications (ITDs) and with tyrosine kinase domain mutations and has been shown to inhibit other kinases implicated in diseases, including

KIT in advanced systemic mastocytosis (advSM) (Andrejauskas-Buchdunger and Regenass, 1992; Fabbro et al., 2000; Propper et al., 2001; Weisberg et al., 2002; Gotlib et al., 2005; Stone et al., 2005; Barry et al., 2007). Midostaurin is in clinical development for treatment of FLT3-mutated AML and advSM (Stone et al., 2005; Fischer et al., 2010; Gallogly and Lazarus, 2016; Gotlib et al., 2016). Based primarily on the phase 3 RATIFY clinical study (Stone et al., 2015), the US Food and Drug Administration granted approval to midostaurin in April 2017.

Midostaurin is rapidly and almost completely absorbed in humans after oral administration (Yin et al., 2008; Dutreix et al., 2013) and is highly distributed. Midostaurin is extensively metabolized by CYP3A4 to form its two metabolites, CGP52421 (7-hydroxylation, epimer 2) and CGP62221 (O-demethylation) (Yin et al., 2008; Wang et al., 2008). Elimination is primarily through fecal excretion, mostly as metabolites. CGP52421 and CGP62221 have also been identified as being pharmacologically active

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ABBREVIATIONS: 4 β HC, 4 β -hydroxycholesterol; ADME, absorption, distribution, metabolism, and excretion; advSM, advanced systemic mastocytosis; AGP, α 1-acid glycoprotein; AML, acute myeloid leukemia; AUC, area under the curve; Caco-2, continuous heterogeneous human epithelial colorectal adenocarcinoma cell line; CL, clearance; CL/F, apparent oral clearance; CL_{int}, intrinsic clearance; DDI, drug-drug interaction; F_a, fraction of dose absorbed; F_g, fraction of the dose that escapes presystemic intestinal first-pass elimination; FLT3, fms-like tyrosine kinase 3; FMI, final market image; fu_{gut}, fraction of unbound drug in the gut; GM, geometric mean; GMR, geometric mean ratio; HLM, human liver microsome; inf, infinity; ITD, internal tandem duplication; IL-6, interleukin 6; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetics; Pop, population; Q, intercompartmental clearance; TDI, time-dependent inhibitor; t_{lag}, lag time; V_{sac}, volume of distribution for the single adjustable compartment; V_{ss}, volume of distribution at steady state.

against cells expressing *FLT3*-ITDs and *KIT* D816V (Propper et al., 2001). Midostaurin (EC_{50} of cytotoxicity response of 39 and 47 nM for *FLT3*-ITDs and *KIT* D816V, respectively) and CGP62221 (EC_{50} of cytotoxicity response of 30 and 70 nM for *FLT3*-ITDs and *KIT* D816V, respectively) showed similar potency (Levis et al., 2006), whereas CGP52421 showed an average approximately 10-fold reduced potency (EC_{50} of cytotoxicity response of 656 and 233 nM for *FLT3*-ITDs and *KIT* D816V, respectively). Both metabolites were also found to be CYP3A4 substrates (Duttreix et al., 2013). Therefore, drug-drug interactions (DDIs) can result in exposure alterations of midostaurin and its metabolites when coadministered with drugs that inhibit or induce CYP3A4. Furthermore, midostaurin and its two active metabolites were reversible and time-dependent inhibitors (TDIs) and inducers of CYP3A4 in vitro. These mixed CYP3A4 interactions can potentially affect the exposure of victim drugs when coadministered with drugs that are sensitive CYP3A4 substrates (e.g., midazolam). Due to these complex DDI mechanisms, midostaurin and its metabolites can act as CYP3A4 substrates, inhibitors, and inducers and also affect their own metabolic clearance (CL) (Fahmi et al., 2009; Fenneteau et al., 2010; Reitman et al., 2011; Rowland Yeo et al., 2011; European Medicines Agency, 2012; Food and Drug Administration, 2012; Garg et al., 2012; Prueksaritanont et al., 2013) or that of other drugs.

In the initial phase of development, DDI studies were conducted in healthy volunteers to address the clinical relevance of CYP3A4-related DDIs, with midostaurin as either a victim of a ketoconazole or rifampicin interaction or a perpetrator of midazolam (Duttreix et al., 2013, 2014). However, these early clinical studies were conducted mostly using relatively short dosing periods. The terminal half-lives of midostaurin, CGP62221, and CGP52421 in plasma are 19.6, 32.2, and 482 hours, respectively. Due to the complex DDI mechanisms of midostaurin and its metabolites, it is important to evaluate potential DDIs under steady-state conditions to support clinical recommendations and potential product label language. Because such DDI studies are not feasible in healthy subjects, we aimed to use a multipronged approach to investigate the net effect of steady-state midostaurin on CYP3A4 activity. To achieve this goal, we did the following: 1) built a physiologically based pharmacokinetic (PBPK) model to describe the pharmacokinetics (PK) of midostaurin and its two active metabolites using a top-down approach leveraging prior clinical PK data in both healthy subjects and patients; 2) verified the performance of the model in predicting clinical PK profiles of midostaurin and its metabolites, particularly in simulating clinically observed DDIs under single-dose conditions; and 3) used the model to predict CYP3A4-related DDIs with midostaurin at steady state. Furthermore, the utility of the endogenous plasma biomarker 4 β -hydroxycholesterol (4 β HC) as a tool for assessing CYP3A4 activity was explored to address the uncertainty in evaluating the net effect of CYP3A4 induction or inhibition of new molecular entities that have mixed inhibition or induction DDI mechanisms in vitro. The final model was also used to predict DDI potential with other CYP3A4 substrates, inhibitors, and inducers; the model limitations are discussed. The prediction results from our case example provide a basis for optimal clinical study recommendations and potential label language.

Materials and Methods

PBPK modeling was accomplished following these general steps: 1) the model was initially developed using physiochemical data; absorption, distribution, metabolism, and excretion (ADME) data; and in vitro DDI study data and optimized to fit the clinical PK data of the parent and its two metabolites; 2) the model was verified by comparing simulated data with data from clinical DDI studies; and 3) the model was applied to predict potential DDI effects of CYP3A4 inhibitors or inducers on midostaurin and its metabolites as well as effects on midazolam under steady-state conditions. Finally, the results from plasma 4 β HC levels (fold change

relative to baseline) were used to confirm the net effect of time-dependent inhibition/induction of CYP3A4 by midostaurin and its metabolites.

PBPK Model Development in Healthy Subjects

A PBPK model was built using Simcyp Simulator, version 15, release 1 (Certara, LP, Princeton, NJ) for midostaurin and its two metabolites, CGP52421 and CGP62221, using in vitro ADME and in vivo PK data. The key input parameters are summarized in Table 1, and described here.

Physiochemical Properties and Plasma Binding. The molecular weights of midostaurin, CGP52421, and CGP62221 are 570.6, 586.6, and 556.6 g/mol, respectively, and the water partition coefficient (logP octanol:water) ratios used were 5.5, 4.8, and 4.7 (calculated logP values), respectively. The compound type was entered as a monoprotic base, with a pK_a value of 11.2 for midostaurin and CGP62221 and a pK_a value of 10.8 for CGP52421 (predicted using ADMET Predictor version 6.5; Simulations Plus, Lancaster, CA). The blood-to-plasma ratios for midostaurin, CGP62221, and CGP52421 were entered as 0.55 based on the internal experimental values.

The fractions bound to plasma for all three components were very high (>99%). For these very highly bound compounds, an equilibrium gel filtration method was used (Weiss and Gatlik, 2014; He et al., 2017). Midostaurin was mainly bound to human α 1-acid glycoprotein (AGP) according to an internal study. Therefore, AGP was selected as a plasma-binding component in the model.

Absorption. A first-order model for midostaurin absorption from the gut lumen was chosen. Several key input parameters were applied. First, the fraction of dose absorbed (F_a) was entered as 0.85, primarily to optimize the C_{max} , based on the observed data from a single oral 50-mg dose of midostaurin in a prior clinical study. This F_a value was consistent with the moderate to high absorption observed in preclinical species and humans (He et al., 2017). The absorption rate constant and lag time were user defined as 1.5 1/h and 0.3 hours, respectively, to optimize C_{max} and the time at which C_{max} is reached that were observed in several clinical trials (after a single dose). Second, the fraction of unbound drug in the gut (f_{ugut}) was predicted to be 0.3, and, accordingly, by Simcyp, the output fraction of the dose that escapes presystemic intestinal first-pass elimination (F_g) value was 0.15, a value derived from the Simcyp-predicted f_{ugut} value. This predicted human F_g value was consistent with the observed rat F_g value based on calculations from data obtained in a rat in vivo study (data not shown). Finally, the nominal flow through the gut value captures the fact that a higher permeability past the enzyme will decrease first-pass exposure to the enzyme, as will a greater blood flow carrying the drug away from the enterocytes. The value was predicted to be 5.23 l/h with Simcyp, using the apparent permeability data from an internal continuous heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2) study.

Distribution. The minimal PBPK model in Simcyp was used with a single adjusting compartment for all three components. The volume of distribution at steady state (V_{ss}) was estimated to be 1 l/kg, with parameters for a intercompartmental CL (Q) of 3 l/h and a volume of distribution for the single adjustable compartment (V_{sac}) of 0.82 l/kg. The estimated midostaurin oral V_{ss} value was close to the observed geometric mean (GM) or median apparent volume of distribution values, which ranged from 1.0 to 1.3 l/kg. For CGP52421, the V_{ss} value was estimated to be 1.8 l/kg, with a Q of 10 l/h and a V_{sac} of 1.3 l/kg. For CGP62221, the V_{ss} value was estimated to be 1.3 l/kg, with a Q of 2 l/h and a V_{sac} of 1.1 l/kg. All values were estimated based on optimization to best fit to PK data from clinical trials (Supplemental Table 9).

Elimination. The retrograde model was used to calculate in vitro intrinsic CL (CL_{int}) values of the relevant metabolizing enzymes from intravenous or apparent oral CL (CL/F). In brief, the CL/F of 2.4 l/h was estimated to optimize the plasma concentration-time profiles of midostaurin observed in the clinical trials used for model development. This estimated oral CL value was consistent with the observed median or GM CL/F values, which ranged from 2.1 to 3.8 l/h. The fraction metabolized by CYP3A4 was set to a value of 1 for midostaurin based on human in vitro findings and the human ADME study. As such, the CYP3A4 CL_{int} value was calculated to be $\sim 25.2 \mu\text{l}/\text{min}/\text{mg}$ per picomole CYP3A4 by the retrograde model for enzyme kinetics in Simcyp. In a second step, the CYP3A4 CL_{int} was divided between the involved elimination pathways (i.e., CGP52421, CGP62221, and other CYP3A4-mediated pathways). The fraction of CYP3A4 for the metabolism (formation of CGP52421 and CGP62221)/elimination of midostaurin was estimated. The estimations are based mainly on an enzyme

TABLE 1
Input parameters for midostaurin, CGP52421, and CGP62221

Parameter	Midostaurin		CGP52421		CGP62221	
	Value	Reference	Value	Reference	Value	Reference
mol. wt. (g/mol)	570.6		586.6		556.6	
logPo:w	5.49	Internal database	4.76	Internal database	4.68	Internal database
pKa	11.2	Predicted ^a	10.8	Predicted ^a	11.2	Predicted ^a
Compound type	Monoprotic base					
f _{up}	0.00015	Internally measured	0.00021	Internally measured	0.00038	Internally measured
B/P	0.55		0.55	Assumed same as parent	0.55	Assumed same as parent
Plasma-binding component	AGP		AGP		AGP	
First-order absorption						
F _a	0.85 (0.65, advSM)	User defined				
k _a (h ⁻¹)	1.5 (0.683, advSM)	(optimized)				
Lag time (h)	0.3					
Q _{gut} (l/h)	5.23	Simcyp predicted				
f _{u, gut}	0.3					
Pe _{ff, man} (10 ⁻⁴ cm/s)	0.835					
Caco-2 (10 ⁻⁶ cm/s)	1.4	Internally measured				
Caco-2 reference (10 ⁻⁶ cm/s)	17					
Minimal with single adjusting compartmental distribution						
Q (l/h)	3 (2.889, AML/advSM)	Estimated from clinical PK data (a single-dose trial)	10	Estimated from clinical PK data (a single-dose trial)	2	Estimated from clinical PK data (a single-dose trial)
Volume (V _{sac} [l/h])	0.82 (0.623, AML/advSM)		1.8		1.1	
V _{ss} (l/h)	1 (0.742, AML/advSM)		1.3		1.3	
Elimination						
Hepatic CL by CYP3A4 (%)	100	Estimated from human ADME study	90	Estimated from human ADME study	90	Estimated from human ADME study
CL _{int} , CYP3A4 formation of CGP52421 (μl/min per picomole CYP3A4)	9.3 (3.9, AML/advSM)	See Elimination				
CL _{int} , CYP3A4 formation of CGP62221 (μl/min per picomole CYP3A4)	9.3 (3.9, AML/advSM)					
CL _{int} , CYP3A4 formation of other metabolites (μl/min per picomole CYP3A4)	6.6 (2.8, AML/advSM)		3.18 (0.557, AML patients)		1.19	
Additional HLM CL (μl/min per milligram protein)			48.4 (6.48, AML patients)		18.1	
Active uptake into hepatocytes	1	Default				
CL _R	0	See Elimination				
CYP3A4-related interaction						
Reversible inhibition CYP3A4 K _{iu} (μM)	0.25	Internally measured (Supplemental Table 2)	0.44	Internally measured (Supplemental Table 2)	0.25	Internally measured (Supplemental Table 2)
TDI K _I , total (μM) k _{inact} (1/h)	1.02 2.8	Internally measured (Supplemental Table 3)	1.97 3.0	Internally measured (Supplemental Table 3)	2.01 4.62	Internally measured (Supplemental Table 3)
Induction of CYP3A4 Ind _{max} (fold) IndC ₅₀ (μM)	9.14 0.0026	Internally measured (Supplemental Table 4) and optimized	10.8 0.0053	Internally measured (Supplemental Table 4) and optimized	11.97 0.0027	Internally measured (Supplemental Table 4) and optimized

B/P, blood-to-plasma ratio; CL_R, renal CL; f_{up}, fraction of unbound drug in plasma; Ind₅₀, half-maximum induction rate; IndC₅₀, concentration of inducer at half-maximal induction; Ind_{max}, maximal fold induction over vehicle control; k_a, absorption rate constant; K_I, concentration producing a half-maximal rate of activity reduction; k_{inact}, maximal rate of activity reduction; K_{iu}, unbound inhibition constant; logPo:w, water partition coefficient; Pe_{ff, man}, effective permeability in man; Q_{gut}, nominal flow through the gut.

^aDetermined using ADMET Predictor version 6.5 (Simulations Plus).

phenotyping study using human liver microsomes (HLMs) and cytochrome P450-selective chemical inhibitors; the estimations showed that CYP3A4 contributed to most of the hepatic oxidative microsomal metabolism of midostaurin. Furthermore, CGP52421 and CGP62221 are also metabolized mainly by CYP3A4. The human ADME study (He et al., 2017) showed that midostaurin undergoes extensive metabolism and is predominantly excreted through feces, mostly as metabolites. The fractions of CYP3A4 for the metabolism/elimination of midostaurin in human fecal excreta were estimated to be 37%, 37%, and 26% for CGP52421, CGP62221, and other minor

metabolites, respectively. Consequently, the values of CL_{int} in microliters per minute per picomole CYP3A4 were assigned to the following CYP3A4 pathways (in μl/min per picomole CYP3A4): CGP52421, 9.3; CGP62221, 9.3; other CYP3A4, 6.6.

For the oral CL/F, the values were estimated to be 0.4 and 0.27 l/h for CGP52421 and CGP62221, respectively, based on optimization to best fit to the plasma concentration-time profiles of these metabolites from clinical trials. Furthermore, it was assumed that approximately 90% of CGP52421 and CGP62221 CL was mediated by CYP3A4, because they were detected at low

levels in human excreta. The CYP3A4 CL_{int} values derived from the CL/F were predicted by the retrograde model to be 3.18 $\mu\text{L}/\text{min}$ per picomole CYP3A4 and 1.19 $\mu\text{L}/\text{min}$ per picomole CYP3A4 for CGP52421 and CGP62221, respectively.

Data from the human ADME study indicated that midostaurin in urine was not detected, as shown in a high-performance liquid chromatography profile of radioactive components in pooled urine (0–72 hours). Therefore, the renal CL was assumed to be negligible and set to 0 L/h .

Interaction. The potential of midostaurin, CGP52421, and CGP62221 to inhibit human CYP3A4/5 enzyme activity and act as CYP3A4/5 TDIs was assessed using pooled HLMs (Supplemental Material). The potential for midostaurin, CGP52421, and CGP62221 to act as inducers of CYP3A4/5 enzymes was also evaluated in primary human hepatocytes of three individual donors using both mRNA quantification (real-time polymerase chain reaction) and CYP3A4/5 activity measurements (Supplemental Material). All of the *in vitro* enzyme activity was determined using liquid chromatography-tandem mass spectrometry of selective CYP3A4/5 probe substrate metabolism (Supplemental Material). The input parameters used for the induction of CYP3A4, TDI, and reversible inhibition of CYP3A4/5 are summarized in Table 1.

Model Assumptions. Several assumptions were made for the projection of human PK parameters. First, human F_a was set to 0.85 (see *Absorption*). Next, the fractions of total metabolism catalyzed by CYP3A4 in humans were estimated to be fractions of total CGP52421 metabolism catalyzed by CYP3A4 of ~0.37, fractions of total CGP62221 metabolism catalyzed by CYP3A4 of ~0.37, and fractions of total other metabolite metabolism catalyzed by CYP3A4 of ~0.26, with the assumptions that metabolite epimer structures were similar and that sequentially formed metabolites were equally contributed by primary and secondary metabolism. The CYP3A4/5 TDI total concentration producing a half-maximal rate of activity reduction (total K_i) was used. The CYP3A4 mRNA induction EC_{50} values of midostaurin, CGP52421, and CGP62221 were entered and optimized in the models (i.e., 20-fold more potent than the experimental value). These modifications (i.e., lower EC_{50} and fixed maximal induction) were judged to be appropriate based on the very high plasma protein binding of all three components observed (>99%); lower intracellular free concentrations in hepatocytes would be expected, which could reflect the amount of drug available for binding to the pregnane X receptor. The model was developed and verified using data from clinical studies in healthy subjects (further details follow). The disease- and/or age-related physiologic changes affecting drug ADME properties, including enzyme activity and function, were not considered. Finally, the transporter-related drug interaction property was not included in the model.

Population. The simulations were performed using a simulated healthy volunteer population (Pop) built in the Simcyp simulator, with individuals who were 20–55 years of age; the proportion of female subjects was set to 50%. The Pop size was 100, with 10 trials of 10 subjects per trial.

Simulation Trials. The simulated trials were run in a fasted state. The simulated trials for plasma PK of midostaurin and its two metabolites after single and multiple twice-daily doses are presented as follows. First, observed and predicted PK parameters and plasma concentration-time profiles of midostaurin, CGP52421, and CGP62221 after a single dose of midostaurin with a final market image (FMI) formulation of 50 mg in the simulated subjects were compared with those in healthy volunteers in clinical studies. Control arms included food effect, relative bioavailability, DDIs with ketoconazole, and DDIs with rifampicin. Second, observed and predicted PK parameters and plasma concentration-time profiles of midostaurin, CGP52421, and CGP62221 after midostaurin (FMI) 75 mg twice daily on days 1 and 2 and 75 mg daily on day 3 in the simulated subjects were compared with those in healthy volunteers in a cardiac intervals investigation. Third, observed and predicted PK parameters and plasma concentration-time profiles of midostaurin, CGP52421, and CGP62221 after midostaurin (FMI) 50 mg twice daily on days 1–6 and a single dose on day 7 in the simulated subjects were compared with those in healthy subjects with normal hepatic function in a PK study in subjects with impaired hepatic function.

PBPK Model Performance

The performance of the PBPK model was verified by comparing the simulated data with clinically observed DDIs. Input parameters for the PBPK model are in Supplemental Tables 10–15. The ketoconazole, rifampicin, and midazolam models available in the Simcyp compound library were used in the simulations. The simulated trials for plasma PK of midostaurin and its two metabolites in the

presence of CYP3A4/5 inhibitors, a CYP3A4 inducer, or sensitive CYP3A4 substrates are shown in Table 2.

PBPK Model Development in Patients with AML and AdvSM

A PBPK model was also built to simulate the PK of midostaurin and its metabolites in patients with AML and advSM using a top-down approach. The model was mainly adopted from the previous Simcyp model in healthy subjects but with modifications. The assumption of these two patient models was that the protein binding fraction metabolized by CYP3A4 and *in vitro* DDI parameters of midostaurin and its metabolites were the same in patients and healthy subjects. The parameters were updated based on the parameter estimated in the Pop PK of midostaurin in patients with AML and advSM. These values were entered as described in the model for healthy subjects, with the exception of the following changes (Table 1).

For patients with AML, parameters of distribution and elimination for midostaurin and parameters of elimination for CGP52421 were updated in the model for healthy subjects. V_{ss} values of midostaurin were changed from 1 to 0.742 L/kg , with changes in parameters for Q (3–2.889 L/h) and V_{sac} (0.82–0.623 L/kg). CL_{int} of midostaurin was changed from ~25.2 to 10.6 $\mu\text{L}/\text{min}$ per milligram per picomole isoform. Accordingly, the midostaurin value of CL_{int} (in microliters per minute per picomole CYP3A4) was assigned to the following CYP3A4 pathways: CGP52421 (37%) = 3.9; CGP62221 (37%) = 3.9; other (26%) = 2.8. For CGP52421, CL/F and CL_{int} were changed from 0.4 to 0.07 L/h and from 3.18 to 0.557 $\mu\text{L}/\text{min}$ per milligram per picomole CYP3A4, respectively, with additional HLM CL of 6.48 $\mu\text{L}/\text{min}$ per milligram protein. Comparison of PBPK-simulated and Pop PK-simulated data can be found in Supplemental Table 5, indicating that PBPK-simulated area under the curve (AUC) and C_{max} values were within ≤ 2 -fold of the values in the Pop PK data analysis.

For patients with advSM, parameters of absorption, distribution, and elimination for midostaurin were updated in the model for healthy subjects, so that the same V_{ss} and CL_{int} values of midostaurin used in the model for patients with AML were used in the model for patients with advSM. In addition, the midostaurin F_a value was changed from 0.85 to 0.65, and the absorption rate constant was changed from 1.5 to 0.683 L/h . These values were updated based on optimization to best fit to Pop PK data in patients with advSM. Comparison of PBPK-simulated and Pop PK-simulated data can be found in Supplemental Table 6, indicating that PBPK-simulated AUC and C_{max} values were within ≤ 2 -fold of the values in the Pop PK data analysis.

PBPK Model Application

After model verifications using data from clinical PK studies as well as clinical DDIs with ketoconazole, rifampicin, and midazolam in healthy subjects, the model was used to simulate hypothetical DDI scenarios at steady-state levels in healthy subjects and in patients with AML and advSM. These scenarios included 1) predicting the effect of moderate and strong CYP3A4 inhibitors and inducers on the exposure of midostaurin and its metabolites and 2) predicting the effect of midostaurin on the exposure of midazolam.

Simulation of the Effects of Moderate and Strong CYP3A4 Inhibitors and Inducers. The potential effects of CYP3A4 inhibitors fluconazole (moderate), itraconazole (strong), and ketoconazole (strong) and those of inducers efavirenz (moderate) and rifampicin (strong) on the PK of midostaurin and its metabolites were simulated. The Simcyp default PBPK models for efavirenz, fluconazole, and itraconazole (fed capsule) with OH-itraconazole, ketoconazole, and rifampicin were used in these simulations. Next, midostaurin 50 mg twice daily was administered for 21 days; efavirenz 600 mg daily, fluconazole 200 mg daily, itraconazole 100 mg twice daily, ketoconazole 400 mg daily, or rifampicin 600 mg daily was coadministered with midostaurin for 7 days, starting on day 22. The effects of inhibitors and inducers on the PK of midostaurin were assessed after the last midostaurin dose on day 28.

Simulation of the Effects of Midostaurin and Its Metabolites on Midazolam. Due to a technical limitation in the current version of Simcyp, the model does not have the ability to enter midostaurin with two metabolites as perpetrators. Therefore, an alternative approach was used in which the simulations were performed using the perpetrator as the substrate of the model and the victim as the inhibitor of the model. Briefly, two simulations were conducted with midostaurin and its two metabolites in the substrate position and midazolam in the inhibitor position. In the first simulation, the DDI properties of midostaurin and its

TABLE 2
Summary of trial simulation design

	Simulated Pop	Observed Pop	Dosing Regimen	PK Measurement of Substrate
Model performance of midostaurin PK and ketoconazole DDIs	Healthy volunteer	Healthy volunteer	Ketoconazole 400 mg daily on days 1–10 + midostaurin (FMI) 50 mg on day 6	0–120 h, 0–inf
Model performance of midostaurin PK and rifampicin DDIs	Healthy volunteer	Healthy volunteer	Rifampicin 600 mg daily on days 1–14 + midostaurin (FMI) 50 mg on day 9	0–144 h, 0–inf
Model performance of midazolam PK and midostaurin-midazolam DDIs	Healthy volunteer	Healthy volunteer	Midazolam 4 mg daily on days 1 and 6 + midostaurin 100-mg single dose on day 1, 50 mg twice daily on days 2–4	Day 1: 0–10 h, 0–inf Day 6: 0–10 h

metabolite CYP3A were turned off, representing the control PK of midazolam (in the absence of interaction). The second simulation was performed in the presence of midostaurin by including all the DDI properties, representing the interaction between midazolam as a perpetrator on midostaurin and its metabolites as victims. The exposure parameters (e.g., AUC and C_{max}) of midazolam from the first simulation were compared with those of the second simulation to determine the effect of midostaurin and its metabolites on midazolam. The dose regimen was midazolam 4 mg on day 28, with midostaurin 50 mg twice daily on days 1–28.

Plasma 4βHC Measurement

Study Design. Healthy adult volunteers 18–55 years of age were randomized to receive either rifampicin 600 mg (positive control) or placebo (negative control) from day 1 to 14. All individuals received midostaurin 50 mg on day 9. This unique, single administration was deemed not to interfere with the rifampicin-placebo effect. The positive control arm ($n = 20$) and negative control arm ($n = 20$) served as references for the progression of 4βHC levels. In a separate study, data from patients with advSM enrolled in an open-label, phase 2 study who received midostaurin 100 mg twice daily for 28 days ($n = 10$) were used to determine the 4βHC concentration profile. The study was conducted in accordance with the Declaration of Helsinki and approved by all relevant institutional review boards and ethics committees. The study design is summarized in Table 3.

Sample Collection and Analysis. To evaluate the plasma concentration of 4βHC, peripheral blood samples were collected on days 1 (baseline), 9 (before receiving midostaurin), 11, and 15 in the positive and negative control arms (healthy participants in a clinical DDI study with rifampicin) and on days 1, 3, 8, 15, 22, and 28 of cycle 1 in the patient study. The plasma concentration of 4βHC was measured as described (Diczfalusy et al., 2011), using validated liquid chromatography-tandem mass spectrometry with a lower limit of quantification of 3 ng/ml.

Statistical Analyses. Statistical analyses were descriptive only; no formal statistical tests were performed. Only subjects with an available full 4βHC profile were included in the analysis. For each subject, values at each time point and the percentage change from baseline were considered and median values were

computed (data not shown); the mean and GM of the individual value from baseline were also calculated. Results were plotted to visually represent the data and support the interpretations.

Results

Simulated and Observed PK after Single and Multiple Doses of Midostaurin in Healthy Subjects

A Simcyp model was established to predict the PK of midostaurin, CGP52421, and CGP62221 after single (50 mg) and multiple (50 or 75 mg) oral doses in healthy subjects. Simulated PK profiles were compared with data from several clinical trials in which single-agent PK data were available. Overall, the PK of midostaurin, CGP52421, and CGP62221 were predicted reasonably well. The simulated mean plasma concentration-time profiles with observed data overlaid are shown in Fig. 1. The representative tabulated simulated and actual PK parameters for midostaurin, CGP52421, and CGP62221 after a single dose of 50 mg and 50 mg twice daily of midostaurin can be found in Supplemental Tables 7 and 8.

Simulated and Observed DDIs with CYP3A4 Inhibitor Ketoconazole and Inducer Rifampicin in Healthy Subjects

The clinical DDI trial of midostaurin 50 mg with ketoconazole 400 mg daily or rifampicin 600 mg daily was simulated according to the clinical trial design in healthy subjects. Simulated profiles for the interaction of midostaurin and ketoconazole or midostaurin and rifampicin were then compared with observed data, as shown in Fig. 2.

The predicted and observed midostaurin AUC GM ratios (GMRs) were 5.8 (AUC_{inf} , 6.3) and 6.1 (AUC_{inf} , 10.4), respectively, and the predicted and observed C_{max} GMRs were 2.1 and 1.8, respectively, when midostaurin was coadministered with ketoconazole. For

TABLE 3
4βHC STUDY design (CPKC412A2110; registered as EudraCT no. 2009-009895-11)

Pop	Study Arm	Treatment	Screening (Days –14 to –1)	Study Day													
				Baseline (1)	2	3	4–7	8	9	10	11	12–14	15	16–21	22	23–27	28
Healthy volunteers	Negative control ($n = 20$)	Placebo daily		a	a	a	a	a	a	a	a	a					
	Positive control ($n = 20$)	Midostaurin 50 mg twice daily		c	c	c	c	c	c	c	c	c					
	PK assessment	Rifampicin 600 mg daily							b								
Patients with advSM	Midostaurin arm ($n = 10$)	Midostaurin 50 mg twice daily		d					d		d		d				
	Midostaurin arm ($n = 10$)	Midostaurin 100 mg twice daily		b	b	b	b	b	b	b	b	b	b	b	b	b	b
	PK assessment	4βHC		e		e		e					e		e		e

^aReceipt of placebo.

^bTreatment with midostaurin.

^cTreatment with rifampicin (a CYP3A4 inducer).

Blood samples for the assessment of 4βHC in serum were taken as follows:

^dBlood samples for the assessment of 4βHC in serum for healthy volunteers: days 1, 9 (before midostaurin treatment), 11, and 15;

^eBlood samples for the assessment of 4βHC in serum for patients with advSM: days 1, 3, 8, 15, 22, and 28.

Fig. 1. The black lines represent the simulated mean plasma concentration-time profiles of midostaurin (A, D, and G), CGP52421 (B, E, and H), and CGP62221 (C, F, and I). The dashed lines represent the simulated lower 5th and upper 95th percentiles. The symbols and error bars represent the observed mean plasma concentration-time data and S.D. from two clinical trials (A2111 and A2108) and two control trials (A2109 and A2110) for (A, B, and C). All four trials were open-label, randomized, parallel-group studies in healthy volunteers. (A, B, and C) show day 1 after treatment with midostaurin 50 mg; (D, E, and F) show days 1–3 after treatment with midostaurin 75 mg twice daily for 3 days; and (G, H, and I) show days 1 and 7 after treatment with midostaurin 50 mg twice daily for 6 days and a single dose on day 7.

CGP52421, the predicted and observed GMRs were 0.6 and 1.2 for AUC and 0.4 and 0.5 for C_{max} , respectively. For CGP62221, the predicted and observed GMRs were 0.6 and 1.0 for AUC and 0.3 and 0.6

for C_{max} , respectively. When midostaurin was coadministered with rifampicin, the predicted versus observed decrease in GM AUC and C_{max} for midostaurin was 90% versus 94% and 79% versus 73%,

Fig. 2. The simulated mean plasma concentration-time profiles of midostaurin after treatment with midostaurin 50 mg on day 6 in the absence (solid line) or presence (dashed line) of ketoconazole 400 mg once daily on days 1–10 (A) and on day 9 in the absence (solid line) or presence (dashed line) of rifampicin 600 mg once daily on days 1–14 (B). The symbols (with and without interaction are shown in red and purple, respectively) and error bars represent the observed mean plasma concentration-time data and S.D., respectively.

respectively. The predicted versus observed decrease in GM AUC for CGP52421 was 65% versus 59%, respectively, and for CGP62221, 55% versus 92%, respectively. The corresponding predicted versus observed decrease in GM C_{max} for CGP52421 and CGP62221 was 9% versus 35% and 37% versus no change, respectively. Overall, the model predicted the exposure change in midostaurin reasonably well when it was coadministered with ketoconazole or rifampicin. However, for its metabolites, there was a trend toward underprediction of exposure change compared with observed data (Table 4).

Simulated DDIs with Moderate and Strong CYP3A4 Inhibitors and Inducers at Steady State in Healthy Subjects and Patients with AML and advSM

The potential effects of CYP3A4 inhibitors fluconazole (moderate), itraconazole (strong), and ketoconazole (strong) as well as those of CYP3A4 inducers efavirenz (moderate) and rifampicin (strong) on midostaurin exposure at steady-state levels (50 mg twice daily for healthy subjects and patients with AML and 100 mg twice daily for patients with advSM for 28 days) were simulated both in healthy subjects and in patients with AML and advSM.

GMRs of AUC from time zero to the end of the dosing interval at steady-state ($AUC_{0-\tau}$) $AUC_{0-\tau}$ for itraconazole were predicted to be 2.5-, 1.3-, and 1.3-fold for midostaurin, CGP52421, and CGP62221, respectively, using the model for healthy subjects. GMR values of $AUC_{0-\tau}$ for ketoconazole DDIs were predicted to be 5.4-, 1.6-, and 1.7-fold for midostaurin, CGP52421, and CGP62221, respectively. The results indicated that the impact of DDIs after multiple doses of midostaurin (to steady state) with ketoconazole was similar to the impact of DDIs after a single midostaurin dose (Table 4). GMR values of $AUC_{0-\tau}$ for midostaurin with fluconazole were predicted to be 2.7-, 1.6-, and 1.5-fold for midostaurin, CGP52421, and CGP62221, respectively. GMR values of $AUC_{0-\tau}$ for rifampicin DDIs were predicted to decrease by 43%, 30%, and 25% for midostaurin, CGP52421, and CGP62221, respectively. The impact of DDIs after multiple doses of midostaurin (to steady state) with rifampicin appears to be less than the impact of DDIs after a single dose of midostaurin (Table 4). With coadministration of efavirenz, GMR values of $AUC_{0-\tau}$ were predicted to decrease by 8%, 5%, and 4% for midostaurin, CGP52421, and CGP62221, respectively, suggesting a minimal impact on exposure to midostaurin and its metabolites. The overall prediction results are summarized in the forest plots shown in Fig. 3.

GMR values of $AUC_{0-\tau}$ for itraconazole for AML and advSM were predicted to be 2.2- and 2.0-fold, 1.0- and 1.2-fold, and 1.2-fold for

midostaurin, CGP52421, and CGP62221, respectively, applying the models for patients with AML and advSM. GMR values of $AUC_{0-\tau}$ for ketoconazole DDIs for AML and advSM were predicted to be 4.2- and 4.4-fold, 1.1- and 1.5-fold, and 1.5- and 1.6-fold for midostaurin, CGP52421, and CGP62221, respectively. GMR values of $AUC_{0-\tau}$ for midostaurin with fluconazole for AML and advSM were predicted to be 2.5- and 2.6-fold, 1.2- and 1.6-fold, and 1.5- and 1.6-fold for midostaurin, CGP52421, and CGP62221, respectively. GMR values of $AUC_{0-\tau}$ for rifampicin DDIs for AML and advSM were predicted to decrease by 27% and 34%, 13% and 24%, and 21% and 20% for midostaurin, CGP52421, and CGP62221, respectively. With coadministration of efavirenz, GMR values of $AUC_{0-\tau}$ were predicted to decrease by 5% and 3%, 1% and 2%, and 3% and 2% for midostaurin, CGP52421, and CGP62221, respectively. The overall prediction results are summarized in the forest plots shown in Fig. 3.

Simulated Hepatic CYP3A4 Dynamics After Treatment with CYP3A4 Inhibitors and Inducers

From a victim DDI perspective, the model reasonably predicted the observed changes in midostaurin exposure with ketoconazole and rifampicin. The qualified model for healthy subjects was then applied to predict the DDI effect with ketoconazole or rifampicin at steady-state levels. In the presence of a strong CYP3A4 inducer (rifampicin 600 mg daily), the ratios (relative to the control arm) of maximal increased hepatic CYP3A4 activity were 4.9 and 1.9 after a single dose and twice daily dosing of midostaurin (steady state), respectively (Fig. 4A). These ratios are consistent with predicted reductions of midostaurin exposure by 90% (single dose) and 43% (steady state) due to the change in CYP3A4 enzyme activity. Conversely, in the presence of a strong CYP3A4 inhibitor (ketoconazole 400 mg daily), the ratios (relative to the control arm) of maximal increased hepatic CYP3A4 activity were 1.08 and 1.02 after a single dose and twice-daily dosing of midostaurin (steady state), respectively (Fig. 4B). These ratios are consistent with predicted similar increases of midostaurin exposure by 5.8-fold (single dose) and 5.4-fold (steady state) due to the similar change in CYP3A4 enzyme activity.

Simulated and Observed DDIs with CYP3A4 Substrate Midazolam

The clinical combination trial for a single dose of midazolam (4 mg) with a single dose of midostaurin (100 mg on day 1, followed by 50 mg twice daily on days 2–4) was simulated according to the clinical trial design. The effect of midostaurin on midazolam $AUC_{0-\infty}$ was predicted to be 1.2-fold (observed, 1.0-fold) and 0.74-fold (observed, 0.95-fold)

TABLE 4
Summary of predicted and observed AUC and C_{max} ratios with ketoconazole and rifampicin

	$AUC_{0-20\text{ h}}$ GMR (90% CI range)		C_{max} GMR (90% CI range)	
	Observed	Predicted	Observed	Predicted
With coadministration of ketoconazole ^a				
Midostaurin	6.1 (5.0–7.5)	5.8 (5.1–6.5)	1.8 (1.6–2.1)	2.1 (1.9–2.2)
CGP52421	1.2 (1.0–1.5)	0.6 (0.5–0.7)	0.5 (0.4–0.6)	0.4 (0.4–0.4)
CGP62221	1.0 (0.8–1.2)	0.6 (0.6–0.7)	0.6 (0.5–0.7)	0.3 (0.3–0.3)
	$AUC_{0-144\text{ h}}$ GMR (90% CI range)		C_{max} GMR (90% CI range)	
	Observed	Predicted	Observed	Predicted
With coadministration of rifampicin ^b				
Midostaurin	0.06 (0.05–0.07)	0.10 (0.09–0.12)	0.27 (0.23–0.31)	0.21 (0.18–0.25)
CGP52421	0.41 (0.36–0.46)	0.35 (0.31–0.40)	0.65 (0.59–0.72)	0.91 (0.84–0.99)
CGP62221	0.08 (0.06–0.09)	0.45 (0.40–0.50)	0.63 (0.56–0.70)	1.02 (0.97–1.09)

$AUC_{0-20\text{ h}}$, AUC over 0–20 h; $AUC_{0-144\text{ h}}$, AUC over 0–144 h; CI, confidence interval.

^aDose: 400 mg daily on Days 1–10 + midostaurin 50 mg on Day 6.

^bDose: 600 mg daily on Days 1–14 + midostaurin 50 mg on Day 9.

Fig. 3. Simulated AUC and C_{max} ratios (with 90% confidence intervals) in healthy volunteers (HVs) and in patients with AML and advSM for midostaurin (A), CGP52421 (B), and CGP62221 (C) when coadministered with ketoconazole, itraconazole, fluconazole, efavirenz, and rifampicin. The open circles and closed squares represent GM C_{max} and AUC ratios, respectively.

Fig. 4. (A) The solid green and red lines represent the simulated CYP3A4 hepatic activity (percentage of baseline) after a 50-mg single dose and a 50-mg twice-daily dose of midostaurin to steady state (day 28), respectively, after treatment with rifampicin 600 mg once daily. (B) The solid green and red lines represent the simulated CYP3A4 hepatic activity (percentage of baseline) after a 50-mg single dose and a 50-mg twice-daily dose of midostaurin to steady state (day 28), respectively, after treatment with ketoconazole 400 mg once daily.

on days 1 and 6, respectively. The corresponding change in midazolam $C_{\max}$ was predicted to be 1.2-fold (observed, 0.8-fold) and 1-fold (observed, 0.9-fold), respectively. These results indicated that no CYP3A4 induction effect could be detected in a relatively short study duration. The study duration was short due to safety concerns for healthy subjects. The observed and simulated plasma concentration-time profiles are presented in Fig. 5.

Simulated DDIs with CYP3A4 Substrate Midazolam at Steady State in Healthy Subjects and Patients with AML and advSM

Simcyp Simulation. The potential interaction of midostaurin with the sensitive CYP3A4 substrate midazolam under steady-state midostaurin dosing conditions was simulated in both healthy subjects and patients with AML and advSM. With coadministration of midostaurin (50 mg twice daily in healthy subjects) to steady state (i.e., 28 days), midazolam AUC_{inf} and $C_{\max}$ ratios (relative to dose alone) were predicted to be 0.59 (decreased by 41%) and 0.78 (decreased by 22%), respectively (Fig. 6). The net decrease in midazolam exposure (AUC_{inf}) in the presence of midostaurin (50 or 100 mg twice daily in AML and advSM, respectively) relative to dose alone was predicted to be 0.60 and 0.54, respectively. Overall, this would be indicative of an induction effect of midostaurin and its metabolites at steady state.

Assessment of CYP3A4 Activity Using Plasma Biomarker 4βHC Levels. At baseline, the GM 4βHC levels were similar among the negative control arm, the positive control arm, and the midostaurin arm despite the absence of global randomization (Table 5).

In the negative control arm (subjects receiving placebo), 4βHC levels remained stable on days 9, 11, and 15. No time dependency in levels was observed. In contrast, 4βHC levels notably increased over time in the positive control arm (subjects treated with rifampicin) on days 9, 11, and 15.

In the midostaurin arm (100 mg twice daily), 4βHC levels moderately increased (~1.8-fold) until day 15, at which point they had approximately doubled relative to baseline. The 4βHC levels remained constant from day 15 to PK steady state of midostaurin (day 28) (Fig. 7). GM 4βHC levels on days 3, 8, 9, 11, 15, 22, and 28 are presented (Table 5). A net increase in plasma 4βHC levels was observed with midostaurin (100 mg twice daily) under steady-state conditions, also suggesting an induction effect of midostaurin and its metabolites at steady state. In the

rifampicin arm (positive control), the 4βHC levels increased 3.2-, 3.5-, and 4.3-fold on days 9, 11, and 15, respectively.

Discussion

In the current study, we present a case example using a PBPK modeling approach to predict the DDIs associated with a mixed net effect (TDI and induction) on CYP3A4 for the parent compound as well as its two major circulating metabolites in healthy subjects and in patients with AML and advSM.

In our modeling approach, in vitro study-determined parameters (Supplemental Material) were initially used. However, the simulated concentration-time profiles after multiple dosing showed net auto-inhibition (data not shown) rather than observed auto-induction. Subsequently, EC_{50} values were optimized (20-fold more potent for all three components) based on the fitting of single- and multiple-dosing PK profiles from clinical PK and DDI trials. The underprediction in induction magnitude using in vitro data could be due to other mechanisms involved in the induction of CYP3A4 mRNA. For example, CYP3A4 could be induced through P53 protein activation and may be relevant for patients with cancer (Harmsen et al., 2009; Elias et al., 2013; Goldstein et al., 2013); tyrosine kinase inhibitors may potentially induce CYP3A4 mRNA involving CAR1 and P53 (Lin et al., 2016). Because the induction was ultimately modeled using a “top-down” approach for midostaurin and its metabolites, any other potential mechanisms of CYP3A4 regulation would be captured in the model. Although a more extensive data set was required, this model is considered to be mechanistic because it allows for the incorporation of changes in CL of midostaurin as a result of its auto-inhibition/induction after multiple dosing. By using the middle-out approach (combining top-down and bottom-up approaches), the model could reasonably capture the acute effects of auto-inhibition and subsequent auto-induction, as predicted by increased exposure of midostaurin on day 7 relative to day 1 followed by a decreasing trend in exposure on day 28. The half-life and time to steady state were taken into account, and a standard dosing regimen (50 mg twice daily for healthy subjects and patients with AML and 100 mg twice daily for patients with advSM for 28 days) was consistently used to closely mimic a potential clinical, steady-state dosing regimen. The model was then used to simulate the PK profiles of midostaurin and its metabolites at steady state and predict the subsequent effects on midazolam exposure.

Fig. 5. The solid lines represent the simulated mean systemic plasma concentration-time profiles of midazolam 4 mg in the absence of midostaurin (A), with a single dose of midostaurin 100 mg on day 1 (B), and with an additional twice-daily dose of midostaurin 50 mg on days 2–4 (C). The dashed lines represent the simulated lower 5th and upper 95th percentiles. The symbols (with and without interaction are shown in red and purple, respectively) and error bars represent the observed mean plasma concentration-time data and S.D., respectively.

Per regulatory guidance (European Medicines Agency, 2012; Food and Drug Administration, 2012), the simulated midazolam AUC ratio is consistent with the classification of midostaurin as a weak CYP3A4 inducer (20%–50% decrease in AUC). The simulated net effect of midostaurin and its metabolites as a weak CYP3A4 inducer was also supported by changes in plasma 4β HHC, an emerging biomarker of CYP3A4 activity (Bodin et al., 2001; Bjorkhem-Bergman et al., 2013; Leil et al., 2014). The net effect was an increase in observed 4β HHC,

suggesting CYP3A4 induction. Based on the relationships among midazolam AUC GMR, rifampicin dose, and plasma 4β HHC level, a framework has been developed recently to classify the CYP3A4 inducer potencies of new chemical entities after 14 days of dosing (Mangold et al., 2016). Using this framework (although not accepted currently by regulatory authorities) and considering the observed increase in plasma 4β HHC in our study (maximal 2-fold increase), midostaurin (100 mg twice daily for 28 days) could be viewed as a moderate CYP3A4 inducer in the worst-case scenario. One caveat to using the plasma 4β HHC biomarker is that the potency classification can be dependent on sample size. Based on the low variability of 4β HHC, it is theorized that for a strong inducer such as rifampicin, there would be at least an 80% probability of detecting a 4β HHC elevation with a sample size of <10 . For detecting moderate and weak inducers, the approximate sample size would be $n = 10$ –25 and $n > 60$, respectively (Leil et al., 2014). Given that the 4β HHC data with midostaurin are based on only 10 patients, the classification of midostaurin as a weak to moderate CYP3A4 inducer is, at best, supportive of the PBPK modeling results and needs further confirmation using a larger sample size. Nonetheless, the 4β HHC biomarker data provided an additional tool to evaluate the net effect of CYP3A4 induction or inhibition by midostaurin and its metabolites at steady state when midostaurin was coadministered with midazolam and increased our confidence in the DDI predictions.

To evaluate the victim DDI potential of midostaurin, the CYP3A4 activity after single and multiple doses of midostaurin needs to be considered. The change in simulated CYP3A4 activity with midostaurin alone and in combination with ketoconazole was similar in both single- and multiple-dose scenarios (Fig. 4B). Based on this observation, CYP3A4 inhibitors are predicted to have a similar impact on midostaurin exposure under single- and multiple-dose conditions.

However, the DDI predictions in the presence of CYP3A4 inducers were found to be highly dependent on single- versus multiple-dose conditions. As expected, CYP3A4 activity was not significantly affected by single-dose midostaurin, but it increased upon multiple dosing. Consequently, when another CYP3A4 inducer (rifampicin) was coadministered, the net increase in CYP3A4 activity was less with a single dose than with multiple midostaurin doses, resulting in a 90% decrease in midostaurin exposure under single-dose conditions versus a 43% decrease under multiple-dose conditions. For these reasons, efavirenz (a moderate CYP3A4 inducer) was predicted to have a minimal impact on midostaurin exposure at steady state (i.e., a decrease of 8%).

It is noticed that the predicted increase in the AUC of midostaurin after coadministration of itraconazole 100 mg twice daily was lower than that with ketoconazole 400 mg daily. This is likely in part due to the metabolism of itraconazole as a CYP3A4 substrate being affected by midostaurin and its metabolites, resulting in the induction of CYP3A4 activity. The exposure of itraconazole in the presence of midostaurin was predicted to be lower than for itraconazole administered alone, and the inhibitory effect of itraconazole metabolites appears to be less potent than that of itraconazole alone.

Our simultaneous PBPK modeling approach takes the first step toward assessing the complex CYP3A4 DDI potential of a parent drug and its two metabolites in healthy subjects. To assess the DDI potential of other CYP3A4 inhibitors/inducers used routinely in patients with cancer, two additional PBPK models were built for patients with AML and advSM. Patients with cancers are different than healthy subjects in terms of demographic and physiologic properties. Moreover, the Pop of patients with cancer is heterogeneous, and the differences in various characteristics for patients with cancer, as well as different types of cancer (e.g., AML and advSM), can affect the ADME and PK of drugs. To fully characterize physiologic differences between healthy subjects and patients with cancer, the development of a cancer Pop file has been

Fig. 6. The lines represent the mean plasma concentration-time profiles of midazolam (A), midostaurin (B), CGP5421 (C), and CGP62221 (D) after coadministration of midazolam 4 mg and midostaurin 50 mg twice daily for 28 days.

shown to be a promising tool for the prediction of PK in patients (Cheeti et al., 2013). However, development, validation, and application of cancer-specific profiles will require more comprehensive data collection. Due to the limited knowledge and understanding of the mechanisms of the PK differences observed in healthy subjects and patients with cancer, two individual compound files were built for patients with AML and patients with advSM; the lower V_{ss} and CL_{int} values of midostaurin for patients with both cancer and lower CL of CGP5421 for AML compared with the model for healthy subjects were applied to the patient models. Lower CL values of midostaurin and CGP5421 in patients with AML than in healthy subjects are likely due to multiple

factors. The mechanism of the differences in these parameters is not clear; however, it may be a difference in midostaurin distribution (although similar plasma protein binding between healthy subjects and patients was observed in ex vivo studies) and metabolic CL between the two Pops. It could also be that the metabolic CL of CGP5421 in patients with AML is different than that in healthy subjects and patients with advSM. In recent years, researchers have suggested that circulating levels of cytokines, including interleukin-6 (IL-6), are significantly higher in patients with AML than in healthy subjects (Meyers et al., 2005; Tsimberidou et al., 2008). Furthermore, the suppression of CYP3A4 by IL-6 in hepatocytes was observed and clinical examples

TABLE 5
Geometric mean 4βHC plasma levels at each time point

	Negative Control (n = 20)	Positive Control (n = 20)	Midostaurin (n = 10; 4 males and 6 females)
GM 4βHC plasma levels, CV % (ng/ml)			
Baseline (day 1)	25.3 (35.5)	22.0 (44.0)	29.2 (43.7)
Day 3	N.A.	N.A.	34.1 (43.7)
Day 8	N.A.	N.A.	41.7 (39.1)
Day 9	23.4 (36.4)	75.0 (32.1)	N.A.
Day 11	25.4 (35.9)	89.5 (33.5)	N.A.
Day 15	23.3 (38.8)	102.7 (27.6)	53.8 (34.7)
Day 22	N.A.	N.A.	51.4 (37.1)
Day 28	N.A.	N.A.	56.0 (35.6)
GM percentage change in 4βHC plasma levels from baseline, CV % (%)			
Day 3	N.A.	N.A.	16.8 (0.1)
Day 8	N.A.	N.A.	42.9 (−10.3)
Day 9	−7.7 (102)	240.3 (−26.9)	N.A.
Day 11	0.4 (101)	306.0 (−23.9)	N.A.
Day 15	−8.1 (109)	366.1 (−37.3)	84.2 (−20.6)
Day 22	N.A.	N.A.	76.1 (−14.9)
Day 28	N.A.	N.A.	91.7 (18.4)

N.A., not applicable.

Fig. 7. Overlays of measured 4βHC profiles with ratios relative to baseline and predicted changes in midazolam AUC ratios affected by midostaurin and its metabolites. Midostaurin, patients with advSM who received midostaurin 100 mg twice daily on days 1–28; Negative control, healthy volunteers who received placebo on days 1–15 and midostaurin on day 9. Positive control, healthy volunteers who received rifampicin 600 mg once daily on days 1–15 and midostaurin on day 9.

Time [Days]	3	8	15	22	28
Predicted midazolam GM AUC ratio with midostaurin	0.84	0.60	0.55	0.54	0.54
Plasma 4βHC GM ratio relative to baseline	1.2	1.4	1.8	1.8	1.9

have been reported showing that CYP3A4-mediated metabolism was affected by cytokine modulators (e.g., elevation of IL-6 level), resulting in reduced CYP3A4 expression (Evers et al., 2013). In addition, a lower F_a value of midostaurin than those in the healthy subject model was used for the advSM model. The reduced overall absorption in patients with advSM could be due to the disease-related abnormal numbers of mast cells in organs, including the gastrointestinal tract (Cardet et al., 2013), which may affect oral absorption.

In conclusion, the PBPK model described here using a middle-out approach reasonably predicted PK profiles of midostaurin and its two metabolites after single and multiple dosing. The simultaneous parent and metabolite modeling approach allowed predictions under steady-state conditions that were not possible to achieve in healthy subjects. The model also captured the apparent effect of mixed TDI/induction mechanisms. Our combined, multipronged approach allowed the extension of findings from single-dose clinical DDI studies and enabled predictions of steady-state DDI effects to be made with improved confidence. The results generated using this integrated approach provide the support of clinical recommendations and potential product label language.

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Authorship Contributions

Participated in research design: Gu, Dutreix, Einolf, and He.

Conducted experiments: Gu, Wang, and Chun.

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